

# Double-cropping annual ryegrass and bermudagrass to reduce phosphorus levels in soil with history of poultry litter application

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Received: 11 July 2008 / Accepted: 13 November 2008 / Published online: 29 November 2008  
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**Abstract** Long-term application of poultry litter may result in excessively high soil phosphorus (P). This field study determined the potential of ‘Coastal’ bermudagrass overseeded with ‘Marshall’ annual ryegrass and harvested for hay to reduce the level of Mehlich-3 extractable P (M3-P) that had accumulated in a Savannah soil due to a 30-year history of broiler litter application to bermudagrass, as well as antecedent litter rates of 0, 4.48, 8.96, 17.9, and 35.8 Mg ha<sup>-1</sup> in 1999–2001. Following the cessation of litter, the plots were overseeded in fall 2001–2003 and fertilized in summer with 268 kg N ha<sup>-1</sup> as NH<sub>4</sub>NO<sub>3</sub>. Applying 8.96 Mg ha<sup>-1</sup> litter significantly elevated M3-P in surface soil (0–15 cm depth) from about 183 to 263 mg kg<sup>-1</sup>. Annual dry matter (DM)

yield and P uptake generally increased as litter rate increased up to 17.9 Mg ha<sup>-1</sup>. Analysis of M3-P at four sampling dates from October 2002 to April 2004 found no significant effect of forage system or its interaction with litter rate, and levels in both systems decreased by about 25, 27, 22, 26, and 29% at the five litter rates, respectively. Ryegrass–bermudagrass significantly increased DM yield and P uptake, but did not translate to reductions in M3-P, as compared to bermudagrass winter fallow. With no further litter additions and five harvests per year, both forage systems removed about 49 kg ha<sup>-1</sup> P with a DM yield of 15 Mg ha<sup>-1</sup> and reduced M3-P by about 26 mg kg<sup>-1</sup> annually. Bermudagrass performance is important in the remediation of high soil P.

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**Keywords** Forage · Bermudagrass ·  
Annual ryegrass · Broiler litter · Nutrient uptake ·  
Soil phosphorus

## Introduction

Nutrient loss from high-density livestock operations contributes to the eutrophication of surface waters (Carpenter et al. 1998; Pierson et al. 2001). Integrated poultry (*Gallus gallus domesticus*) production concentrates large numbers of chickens in the vicinity of hatcheries, feed manufacturing mills, and meat processing plants (Sharpley et al. 2007). Mississippi

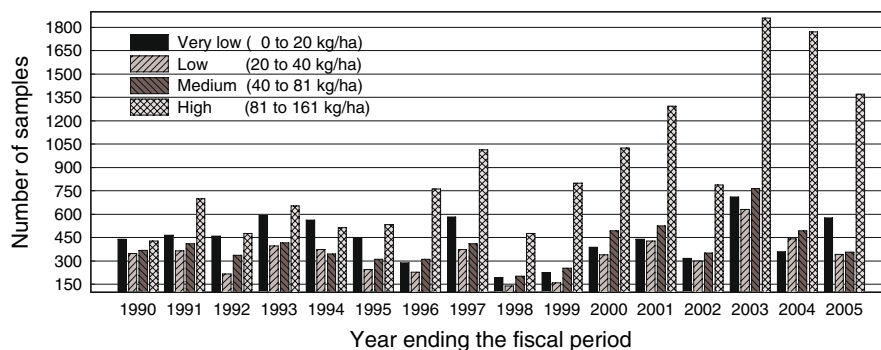
broiler chicken production is located mainly in the south-central region on ~2,000 farms that average 54 ha, and produce about 380,000 broiler chickens annually (USDA Census of Agriculture 2002). Land area available for agricultural crops on 25% of these farms is in the range of 4–20 ha. The chickens are grown in large production houses on smoothed soil floors typically covered with 8–12 cm of wood shavings (Oldham and Coufal 2007). Wet material (termed cake) is removed from the house floor after each flock is removed, commonly 5–6 times annually. Periodically the entire floor covering is removed in a house ‘cleanout’ (Chamblee and Todd 2002; Sistani et al. 2003). This material, called litter (a mixture of manure, wasted feed, and bedding material), is typically applied to pasture and hay crops on the producing farm or nearby farms (Oldham and Coufal 2007). Results of Chamblee and Todd (2002) and Coufal et al. (2006) indicate each ‘average’ farm generates about 345 Mg litter if house cleanout is done biannually, which translates to annual application of 6.4 Mg ha<sup>-1</sup> litter. They estimate 552 Mg litter would be generated if house cleanout is done once each year.

Litter use as fertilizer on producer farms is a common practice; however, imbalances between the quantity of nutrients applied in broiler litter and the relative inefficiency in nutrient utilization by crops contributes to build-up of soil nutrients, particularly P (Gaston et al. 2003; Read et al. 2006). Forage crop production provides a means of removing P from soils and reducing the rate and extent of P accumulation, provided manure is applied when environmental conditions are favorable for plant growth (Brink et al. 2008). Warm-season forages commonly grown in Mississippi require about 263 kg ha<sup>-1</sup> N, 71 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> equivalent P, and 271 kg ha<sup>-1</sup> K<sub>2</sub>O equivalent K to produce 90% of maximum biomass yield (Robinson 1996). Broiler cake and litter, of organic origin, contains all essential elements, but the actual nutrient content varies due to animal diet, litter age, among other factors. Chamblee and Todd (2002) calculated an average N–P<sub>2</sub>O<sub>5</sub>–K<sub>2</sub>O fertilizer equivalent of 2.9–1.5–3 with 19% moisture content (as is basis) based on litter obtained from about 200 Mississippi poultry farms. Their results suggest the average annual addition of nutrients to land total about 289 kg ha<sup>-1</sup> N, 150 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 299 kg ha<sup>-1</sup> K<sub>2</sub>O, assuming all the litter was used on the ‘average’ 54-ha farm without allowance for

buildings, roads, water bodies, or forests. High and continuous application rates are a common practice according to individual producers and University Extension personnel in the poultry production region, and verified by field measurements (Curtis 1998). Consequently, soil test phosphorus (STP, the amount of P<sub>2</sub>O<sub>5</sub> to 15-cm depth) levels on many broiler farms are substantially greater than those required for maximum forage yield (Sharpley et al. 2007; Brink et al. 2008). Analysis of the Mississippi State University Extension Service Soil Testing Laboratory annual summaries for the period 1989–2005 indicates an upward trend in STP in samples submitted for hay and pasture soils in the top 12 poultry producing counties (Fig. 1). This period of time coincides with a doubling of poultry production in Mississippi (data not presented).

The quantity of P released to runoff by soils is influenced by factors other than STP, including mineralogy, form of P species and soil texture (McDowell et al. 2001; Gburek et al. 2005). Because the relationship between dissolved-reactive P in runoff and STP (0–15 cm depth) appeared to depend on when the plots last received litter, Pierson et al. (2001) concluded it may be difficult to predict P loss from STP unless other management factors are known. Mississippi uses the “Phosphorus Index” based planning process to determine the qualitative risk assessment of potential P loss to the environment among different nutrient management scenarios (Oldham 2007; Osmond et al. 2006). Five P source factors, including STP (using Mississippi soil test extractant), are evaluated with the transport factors of erosion, runoff rating, and distance to water (USDA Natural Resource Conservation Service 2007). Phosphorus movement in the landscape depends on soil and site specific hydrological and physiochemical factors that vary in space and time (Sharpley et al. 2007; Sistani et al. 2008). An evaluation of Phosphorus Indices used in the southern United States found the Mississippi index was sensitive to changes in STP, when compared to the other source factors (Osmond et al. 2006).

For sites where broiler litter is being utilized and soil P levels are not high enough to impact surface water quality, producers are encouraged to apply broiler litter based on crop P requirement, as opposed to N requirements (Pierson et al. 2001). Nevertheless, STP will remain high in these soils unless the added P



**Fig. 1** Number of soil samples submitted to Mississippi State University Extension Service Soil Testing Laboratory for pasture and forage recommendations that were classified as having soil test phosphorus (STP, expressed as  $P_2O_5$  equivalents in the 0–15 cm soil depth) levels in the very low (0–18  $kg\ ha^{-1}$ ), low (19–36  $kg\ ha^{-1}$ ), medium (37–72  $kg\ ha^{-1}$ ), and high

(73–144  $kg\ ha^{-1}$ ) indices based on Lancaster P extraction method (Cox 2001). The data represent samples from the top 12 poultry producing counties in Mississippi and were compiled from Annual Reports for the 1989–1990 to 2004–2005 fiscal years

is either tied-up through soil amendments or assimilated by plants. Phytoremediation strategies to minimize the potential risks of high soil P on the environment include (1) substituting fertilizer N additions for litter N to enhance the growth and overall nutrient uptake by forage crops (Evers 2002; Read et al. 2006), and (2) the cessation of litter application and continued harvest and biomass removal until STP returns to a target level (Novak and Chan 2002). Improved hybrid bermudagrass [*Cynodon dactylon* L. (pers.)] has potential to reduce high soil P due to superior biomass yield and high nutrient uptake capacity (Brink et al. 2004). Double-cropping bermudagrass with annual ryegrass (*Lolium multiflorum* Lam.) is a widely used grazing system in the southeastern US, and recent studies suggest it may remediate high soil P through expanding the active growth period and enhanced P removal due to additional hay harvests (Read et al. 2007); however, only the greenhouse study of Gaston et al. (2003) utilized a manure-impacted soil (860 mg P  $kg^{-1}$  in 0–15 cm depth, based on Bray 2 extraction method). Evers (2002) fertilized ryegrass–bermudagrass with broiler litter in fall and different combinations of N fertilizer in spring, and reported ryegrass removed about twice as much P as bermudagrass. Annual ryegrass did not perform as well in studies by Read et al. (2007), as the percentage of total P removed by ryegrass when double-cropped with bermudagrass averaged 39% in 2002 when rainfall was below normal and 8% in 2003 when rainfall was above normal.

The objective of this study was to compare forage biomass production, P uptake, and soil P levels in a ryegrass–bermudagrass (year-round) and bermudagrass (summer) winter fallow system following the cessation of litter application on a manure-impacted Savannah soil. This research was conducted over two experimental periods, each ~3 years in duration. In the first period, five rates of broiler litter were applied to Coastal bermudagrass to determine application management effects on crop nutrient uptake and soil P. The second period, termed the ‘draw off’ phase, examined the potential of bermudagrass overseeded with annual ryegrass and harvested for hay to reduce soil P levels following the cessation of litter application. The present paper focuses on the second period, as results for bermudagrass response to litter rates in the first 3-year period are presented elsewhere (Brink et al. 2008).

## Materials and methods

The study site was a Coastal bermudagrass hay meadow located on a farm near Mize, MS (31°49′N, 89°38′W), on a Savannah fine sandy loam (fine-loamy, siliceous, semiactive, thermic Typic Fragiuult), which consists of moderately drained, strongly acid or very strongly acid soils. A detailed history of litter application was not available, but litter had been applied to the field at ~4.5–9  $Mg\ ha^{-1}\ yr^{-1}$  for the previous 30 years. In April 1999, the bermudagrass sward was cleared of

senesced weeds by mowing, and weed regrowth was controlled using selective herbicides. Soil samples were collected at 0–5 and 5–15 cm depths from 20 cores and composited by depth for chemical analysis (described below). As expected, the initial soil test (0–15 cm depth) showed elevated Mehlich three extractable P (M3-P) of about  $183 \text{ mg kg}^{-1}$  (see Table 5). In Mississippi, crop responses to fertilizer P are not expected when soil test levels exceed  $36 \text{ mg kg}^{-1} \text{ P}_2\text{O}_5$  (or about  $16 \text{ mg kg}^{-1}$  extractable P) based on the Lancaster method (SERA IEG 2007). Although Lancaster-P and M3-P are not easily inter-converted for direct comparison (Cox 2001), the M3-P levels measured at this site are categorized in the very high index by other southern state laboratories that use this method, and thus forage biomass is unlikely to respond to further P amendments (SERA IEG 2007).

During the ‘build-up’ phase (described by Brink et al. 2008), litter rates of 0, 4.48, 8.96, 17.9, and  $35.8 \text{ Mg ha}^{-1} \text{ yr}^{-1}$  (wet weight basis) were applied to small plots ( $4 \text{ m} \times 6 \text{ m}$ ) in a single application on 16 April 1999, 25 April 2000, and 3 May 2001. While the level of nutrients already present in soil suggest the amounts applied in litter would exceed the nutrient requirements of bermudagrass, the rates were chosen to simulate current management practices. In a recent survey of Mississippi broiler producers, Oldham and Coufal (2007) reported a majority (51%) continue to apply litter only to hay or pasture fields. The individual plots were separated by 1-m borders, and replicate blocks were separated by a 2-m wide earthen berm to prevent rainfall runoff from upslope plots. The litter was sampled for pH, the concentration of nine nutrient elements, N P, K, Ca, Mg, Fe, Mn, Cu and Zn, and moisture content, which averaged about 25%. The results are presented by Brink et al. (2008) and were used to calculate the annual rate of nutrients applied in the litter (Table 1). A rate of  $4.48 \text{ Mg ha}^{-1}$  litter is consistent with the P requirement of Coastal bermudagrass, which can remove as much as  $60 \text{ kg ha}^{-1} \text{ P yr}^{-1}$  (Read et al. 2006). Assuming 50% of litter N was available for plant uptake in each growing season,  $8.96 \text{ Mg ha}^{-1}$  would provide half the annual N requirement to produce yields in the range of  $9\text{--}13 \text{ Mg ha}^{-1}$  (about  $269 \text{ kg ha}^{-1}$ ; Brink et al. 2004). Bermudagrass harvests began in late May–early June, and continued at  $\sim 30$ -day intervals depending on rainfall and plant

growth patterns. Plots were harvested five times in 1999, three times in 2000, and four times in 2001 using a sickle-bar mower set at a height of 5 cm.

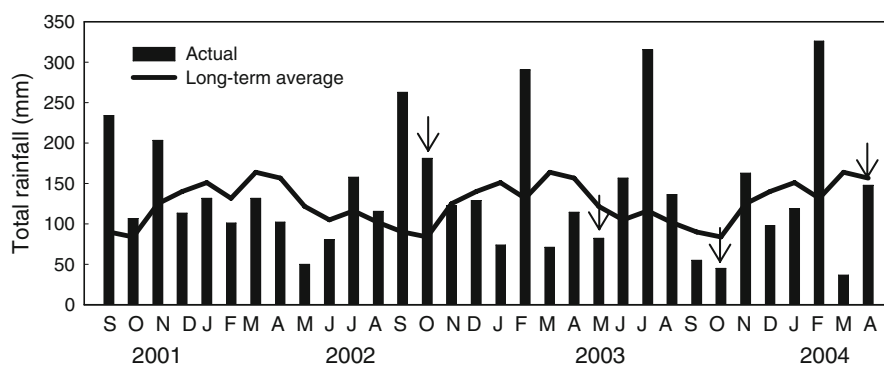
In the present study, which comprised the ‘draw off’ phase, Marshall annual ryegrass was overseeded on 16 October 2001, 1 October 2002, and 9 October 2003. A randomly assigned half of each plot was overseeded at  $28 \text{ kg seed ha}^{-1}$  with a 20-cm row spacing using a Tye<sup>1</sup> drill (The Tye Co., Lockney, TX). All plots were provided  $268 \text{ kg N ha}^{-1}$  (as  $\text{NH}_4\text{NO}_3$ , 34-0-0) in summer 2002 and 2003 using four equal applications of  $67 \text{ kg N ha}^{-1}$  following each harvest date, except the final harvest in October. Rainfall records were obtained from a nearby weather station (Fig. 2). For the cool-season period from October to March, the station recorded 788, 869, and 788 mm rainfall for the 2002, 2003, and 2004 study years, respectively. For the warm-season period the station recorded 770 and 861 mm rainfall in 2002 and 2003, respectively. Annual rainfall was 5–10% above the long-term average of 1,488 mm in 2003. Substantial rainfall was recorded in February 2003 and 2004 (291 and 326 mm, respectively) and in July 2003 (316 mm).

Forage dry matter (DM) yield was determined by cutting a 1- by 6-m swath at a 5-cm stubble height through the center of each plot with a sickle-bar mower. In 2002, the plots were harvested on 25 April, 6 June, 16 July, 15 August, and 1 October. In 2003, the plots were harvested on 3 April, 15 May, 19 June, 7 August, and 19 September. In 2004, the plots were harvested only once on 27 April, when annual ryegrass was in boot stage of development and yield potential of the cool-season forage was highest. The first harvest of each year was a mixture of ryegrass and bermudagrass and no attempt was made to separate species. Subsamples (600–800 g) of the forage were dried at  $65^\circ\text{C}$  for 48 h for determination of percent moisture. Oven-dried forage was ground to pass a 1-mm screen for subsequent chemical analysis (described below). Nutrient uptake was estimated as the product of DM yield and nutrient concentration on each harvest date, and as the summation of values

<sup>1</sup> Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that also may be suitable.

**Table 1** Average annual rate of selected nutrient elements provided by broiler litter that was applied on a wet-weight basis and contained approximately 25% moisture

Litter rate (Mg ha <sup>-1</sup> yr <sup>-1</sup> )	N (kg ha <sup>-1</sup> )	P (kg ha <sup>-1</sup> )	K (kg ha <sup>-1</sup> )	Cu (kg ha <sup>-1</sup> )	Zn (kg ha <sup>-1</sup> )
4.48	120	76	101	2.3	1.6
8.96	241	151	202	4.6	3.3
17.9	482	302	403	9.3	6.5
35.8	965	605	806	18.5	13.1

**Fig. 2** Actual and 30-year average monthly rainfall at Mize, MS, during the duration of the experiment. Arrows indicate months when soil samples were obtained. The weather station was located 2.26 miles west of the study site, and records were obtained from the National Climatic Data Center (NCDC)

across harvests dates to determine total annual nutrient uptake.

To determine litter effects on soil P and the effectiveness of phytoremediation to reduce high soil P, soil samples were obtained in fall and spring. Sampling dates were 2 October 2002, 15 May 2003, 9 October 2003, and 27 April 2004 (Fig. 2). Four, 2.5-cm diameter cores were obtained at the 0–5 and 5–15 cm depths and composited by depth. The samples were placed into zip-lock bags, air dried in a glasshouse, and ground to 0.5-mm size for chemical analyses.

Litter, plant and soil samples were analyzed according to methods described by Brink et al. (2008). Briefly, total N was measured using an automated dry combustion analyzer (Model NA 1500 NC, Carlo Erba, Milan, Italy). Mineral concentration was determined by ashing a 0.8-g subsample in a ceramic crucible at 500°C for 4 h followed by dissolution of the ash in 1.0 ml of 6 M HCl. After 1 h, 50 ml of a double-acid solution of 0.025 M H<sub>2</sub>SO<sub>4</sub> and 0.05 M HCl was added to the crucible, allowed to stand for 1 h, and then filtered through Whatman no. 1 paper. The concentration of nine nutrient elements (P, K, Ca, Mg, Na, Fe, Mn, Cu, and Zn) was determined using emission spectroscopy on an inductively coupled argon plasma optical emission

spectrometer (ICP–OES, Thermo Jarrell Ash Model 1000 ICAP, Franklin, MA). Soil samples were extracted using Mehlich-3 soil extractant (1:10, soil:extractant) and the filtrates analyzed for extractable P and exchangeable K, Cu, and Zn using the ICP (Mehlich 1984). Soil total P was determined by digesting 0.50 g soil with sulfuric acid, hydrogen peroxide, and hydrofluoric acid followed by determination of P using the ICP. Water-extractable P (WEP), which corresponds to solution P and labile P forms (Gburek et al. 2005), was determined by extracting 2 g of soil in 20 ml water for 10 min and ICP analysis of all P forms that pass through a Whatman 2 V filter (~8 µm particle retention, medium-fine porosity).

The experimental design was a split-plot arrangement of treatments with four replicate blocks. Litter rate was the main plot factor, forage system was the split plot factor, and treatments were repeated on the same plot areas each year. Data for total DM yield, nutrient uptake, and soil P were analyzed using SAS mixed model and general linear model (GLM) procedures (Littell et al. 1996; SAS Institute 1999). Because only a single spring harvest was obtained in 2004, data for total DM and nutrient uptake in 2002 and 2003 were used to perform analysis of variance across years. A probability level of  $P \leq 0.05$  was considered significant and treatment means were



compared using Fisher's protected least significant difference (LSD). Soil chemical data were analyzed using values from the 0–5 and 5–15 cm depths, and 'composite' values for the 0–15 cm depth expressed as a weighted-sum average.

## Results and discussion

### Forage yield and nutrient uptake

The effect of antecedent litter rate on total DM yield was significant in 2003, and a similar upward trend was observed in 2002 ( $P < 0.06$ ) (Table 2). The effect of litter rate was not significant in 2004 ( $P > 0.80$ ) when a single harvest was obtained in spring. The litter rate  $\times$  forage system effect was not significant. A yield response to litter can be explained by the additional C and nutrients provided, as well as the additions of N fertilizer in summer (Evers 2002; Read et al. 2006). In 2002, total DM yield in the two forage systems averaged 13.2, 14.6, 15.0, 16.2, and 16.6 Mg ha<sup>-1</sup> at 0, 4.48, 8.96, 17.9, and 35.8 Mg ha<sup>-1</sup> litter, respectively (5% LSD = 2.0). In 2003, total DM yield averaged 15.4, 16.3, 17.5, 18.0, and 20.2 Mg ha<sup>-1</sup> at the five litter rates, respectively (5% LSD = 1.9). The 2–3 Mg ha<sup>-1</sup> increase in annual DM yield in 2003 was associated with high biomass yields on the 7 August harvest date (Table 2), and followed high rainfall amounts in both June and July 2003 (Fig. 2).

In 2002, harvesting annual ryegrass in addition to bermudagrass increased DM yield by about 15, 7, 9, 12, and 0%, respectively, at the five litter rates. Averaged across rates, the additional harvest of ryegrass significantly increased DM yield by 8% (15.7 vs. 14.5 Mg ha<sup>-1</sup>) and P uptake by 16% (59 vs. 51 kg ha<sup>-1</sup>) (Table 2, 3). Overall, a one-time harvest of annual ryegrass in spring removed an additional 2–8 kg P ha<sup>-1</sup> year<sup>-1</sup>, as compared to bermudagrass winter fallow. Low rainfall in 2002 may have limited bermudagrass growth, particularly if ryegrass had depleted much of the soil moisture in spring. This feature may explain results from June 2002, when DM yield was 24–78% lower in the double-cropping system, depending on antecedent litter rate. Bermudagrass is a warm-season species that fixes C by the C4 photosynthetic pathway and thus has a greater potential for biomass production and P removal than

ryegrass, a cool-season species with C3 photosynthesis (Robinson 1996). Although P uptake by forages is a function of P concentration and above-ground biomass yield, the importance of the latter often overrides the first (Brink et al. 2004). Consequently, the high yield potential of bermudagrass coupled with its pronounced yield response to N makes this species an ideal forage crop for phytoremediation of high P soil in the southeastern USA (Novak and Chan 2002; Read et al. 2007). Because a strong correlation was obtained between P uptake and forage biomass at each harvest date (Table 2), improving N fertility and harvest management of annual ryegrass would probably enhance P removal, providing growth of ryegrass in late spring does not reduce bermudagrass yield to the extent that total P removal is decreased (Rowe et al. 2006).

The percentage of annual P removal attributed to annual ryegrass ranged from 20 to 22% across litter rates in 2002 and from 10 to 15% in 2003 (data not shown). This result agrees with Read et al. (2007), but differs from Evers (2002) who reported P uptake by the ryegrass component was about 64–70% of the total P removed by ryegrass–bermudagrass. Difference between the two studies likely resulted from different management practices, as Evers (2002) harvested ryegrass repeatedly beginning in early March and applied 9 Mg ha<sup>-1</sup> broiler litter in fall with a varying number of NH<sub>4</sub>NO<sub>3</sub> (57 kg N ha<sup>-1</sup>) applications in spring. Assuming 60–65% of the N in surface-applied litter was available in the first year (Read et al. 2006), applying litter in fall likely favored annual ryegrass because much of the N mineralized from the litter would be either assimilated or lost by the first of spring and unavailable. The present study and Read et al. (2007) provided N fertilizer in summer, which served to maintain healthy stands of bermudagrass.

Hybrid bermudagrass can remove 50–70 kg P ha<sup>-1</sup> year<sup>-1</sup>, depending on biomass yield and tissue P concentration, which can be influenced by broiler litter rate, N fertility, and soil P concentration (Read et al. 2006; Brink et al. 2008). Harvest removal of P in 2002 and 2003 was greatest when 35.8 Mg ha<sup>-1</sup> litter was used and values were significantly greater at 17.9 than 4.48 Mg ha<sup>-1</sup> litter (Table 3). When 4.48 Mg ha<sup>-1</sup> litter was used, the relatively high P uptake of about 55 kg ha<sup>-1</sup> is consistent with Read et al. (2006) that annual P uptake by Coastal

**Table 2** Forage dry matter (DM) yield at each harvest, total annual DM yield, and the correlation coefficient ( $r$  value) for the relationship between DM yield and P uptake in bermudagrass winter fallow (BGWF) and ryegrass–bermudagrass double cropping (RGBG) systems

Harvest date	0 (Mg ha <sup>-1</sup> yr <sup>-1</sup> )		4.48 (Mg ha <sup>-1</sup> yr <sup>-1</sup> )		9.86 (Mg ha <sup>-1</sup> yr <sup>-1</sup> )		17.9 (Mg ha <sup>-1</sup> yr <sup>-1</sup> )		35.8 (Mg ha <sup>-1</sup> yr <sup>-1</sup> )		SE <sup>a</sup> (Mg ha <sup>-1</sup> )	<i>r</i> value <sup>a</sup>
	BGWF (Mg ha <sup>-1</sup> )	RGBG (Mg ha <sup>-1</sup> )	BGWF (Mg ha <sup>-1</sup> )	RGBG (Mg ha <sup>-1</sup> )	BGWF (Mg ha <sup>-1</sup> )	RGBG (Mg ha <sup>-1</sup> )	BGWF (Mg ha <sup>-1</sup> )	RGBG (Mg ha <sup>-1</sup> )	BGWF (Mg ha <sup>-1</sup> )	RGBG (Mg ha <sup>-1</sup> )		
2002												
25 April	1.15	2.81	1.51	3.39	1.62	3.36	2.67	5.09	2.32	5.31	0.38	0.99
6 June	1.63	1.17	1.66	1.25	1.82	1.34	1.83	1.22	3.00	0.65	0.15	0.99
16 July	3.42	3.59	3.98	3.99	4.36	4.17	4.25	4.60	4.35	3.67	0.28	0.95
15 August	2.93	3.21	3.33	3.12	3.21	3.19	3.33	3.03	3.14	2.88	0.21	0.93
1 October	3.12	3.31	3.60	3.35	3.37	3.59	3.50	3.59	3.50	3.61	0.19	0.95
Total DM	12.2	14.1	14.1	15.1	14.4	15.6	15.6	17.5	16.3	16.1	0.82	0.95
2003												
3 April	0.94	1.57	1.21	2.10	1.31	2.67	1.55	3.67	1.71	5.62	0.23	0.98
15 May	3.02	3.05	3.17	3.18	3.27	3.06	3.80	3.13	3.36	3.08	0.14	0.71
19 June	3.59	2.96	3.74	2.71	4.14	2.86	4.06	2.59	4.30	2.25	0.25	0.96
7 August	5.02	5.49	5.76	5.39	5.51	6.05	5.60	5.29	6.18	5.98	0.29	0.87
19 September	2.42	2.64	2.57	2.71	2.93	3.14	3.17	3.16	3.90	4.06	0.24	0.98
Total DM	15.0	15.7	16.4	16.1	17.2	17.8	18.2	17.8	19.4	21.0	0.68	0.95
2004												
27 April	0.99	1.67	0.89	1.80	0.94	1.62	1.28	2.41	1.32	2.15	0.21	0.98

<sup>a</sup> SE standard error of the treatment mean ( $n = 4$ ) using the experimental error from analysis of variance;  $r$  value, simple correlation coefficient for the relationship between DM yield and P uptake across the five antecedent litter rates, two forage systems and four replicate plots ( $n = 40$ , all  $r$  values are significant at  $P < 0.001$ )

**Table 3** Annual uptake of P by bermudagrass winter fallow and ryegrass–bermudagrass forage systems at five antecedent broiler litter rates and averaged across litter rates

Litter rate and system (Mg ha <sup>-1</sup> yr <sup>-1</sup> )	2002 (kg ha <sup>-1</sup> )	2003 (kg ha <sup>-1</sup> )	2004 (kg ha <sup>-1</sup> )
0	46.3 d	51.7 d	3.5 b
4.48	52.2 cd	57.3 cd	3.7 b
8.96	54.6 bc	62.2 bc	3.6 b
17.9	60.2 ab	64.8 b	5.0 a
35.8	62.6 a	74.5 a	5.0 a
Bermudagrass	51.2 B	61.1 B	3.2 B
Ryegrass–bermudagrass	59.3 A	63.3 A	5.1 A
Rate × system, Pr > F	0.87	0.03	0.45

Within a column, means for litter rate ( $n = 8$ ) and forage system ( $n = 20$ ) followed by a different letter are significantly different at the 5% level according to Fisher's least significant difference

bermudagrass is maximized by the combination of 4.48 Mg ha<sup>-1</sup> litter in April and 202 kg ha<sup>-1</sup> N fertilizer provided by spit applications in May, June, and July. Because P is relatively immobile in soil compared to other major nutrients, its assimilation by plants depends on root function near the soil surface where P and other nutrients often accumulate (Novak et al. 2004; see Table 5). Therefore, decreased growth or function of surface roots under low soil moisture conditions in 2002 may have limited P uptake by bermudagrass, even in soil with excess P. In 2003, P uptake increased as litter rate increased ( $P < 0.01$ ) and a significant litter rate × forage system interaction was

detected (Table 3). Averaged across litter rates, ryegrass–bermudagrass removed 2.2 kg ha<sup>-1</sup> more P than bermudagrass winter fallow. In 2004, a single harvest of ryegrass–bermudagrass significantly increased P uptake by about 2.9 kg ha<sup>-1</sup> or 59%, as compared to bermudagrass winter fallow. Because ryegrass–bermudagrass removed significantly more P, our results demonstrate an advantage of this double-cropping system to modify the balance between inputs and outputs of P in a given field, as well as having a year-round green pasture for either haying or grazing.

#### Soil phosphorus and cropping system

Mehlich-3 extractable P differed significantly across the four sampling dates ( $P < 0.01$ ), decreasing gradually after litter applications ceased (Table 4). Averaged across forage systems ( $n = 8$ ), M3-P levels were significantly lower in October 2003 and April 2004 than in October 2002, and also were lower in May 2003 than October 2002 when 17.9 and 35.8 Mg ha<sup>-1</sup> litter was applied. The observed reductions in M3-P between October 2002 and April 2004 (initial–final) were 52, 61, 58, 87, and 131 mg kg<sup>-1</sup> at antecedent litter rates of 0, 4.48, 8.96, 17.9, and 35.8 Mg ha<sup>-1</sup>, respectively, which corresponds to declines of about 25, 27, 22, 26, and 29% during the study period. Relatively low M3-P levels in May and October 2003 were associated with greater P uptake (Table 3) that approached the maximum of 60 kg ha<sup>-1</sup> P reported for Coastal

**Table 4** Concentration of Mehlich-3 extractable P in surface soil, 0–15 cm depth, sampled at the end of the respective growing seasons in bermudagrass winter fallow (fall 2002 and

2003) and ryegrass–bermudagrass (spring 2003 and 2004) double cropping systems

Litter rate (Mg ha <sup>-1</sup> yr <sup>-1</sup> )	Soil phosphorus (mg P kg <sup>-1</sup> )									
	October 2002		May 2003		October 2003		April 2004		Initial–Final <sup>a</sup>	
	BGWF	RGBG	BGWF	RGBG	BGWF	RGBG	BGWF	RGBG	BGWF	RGBG
0	194	223	201	214	154	182	144	168	49	55
4.48	228	219	213	228	194	193	167	160	62	60
8.96	247	280	239	261	230	242	199	210	47	70
17.9	332	333	289	300	270	302	249	241	82	92
35.8	487	408	387	390	406	364	328	305	159	103
LSD, 5% <sup>b</sup>	62	75	70	44	68	59	53	57	58	79 ns

<sup>a</sup> Reduction in soil test phosphorus based on subtracting final values in April 2004 from initial values in October 2002

<sup>b</sup> LSD, least significant difference for the comparison of litter rate means at the 5% level of probability according to Fisher's protected  $F$  test in analysis of variance; otherwise, not significant (ns)



bermudagrass in Savannah soil (Read et al. 2006). Relatively large reductions in M3-P following the cessation of 17.9 and 35.8 Mg ha<sup>-1</sup> litter is consistent with Read et al. (2007) for a Ruston soil (fine-loamy, siliceous, thermic Typic Paleudult) with no long-term history of litter application. In that study, M3-P levels in plots previously provided 8.96 Mg ha<sup>-1</sup> litter were reduced by about 25 mg kg<sup>-1</sup> in 2003 (a year with above-average rainfall) in the double-cropping system that removed about 42 kg ha<sup>-1</sup> P in five harvests. Gaston et al. (2003) found reductions in soil P (by Bray two extraction methods) were consistent with the total P removed in several hay harvests. They reported soil P decreased by about 73 mg kg<sup>-1</sup> in six harvests of Marshall annual ryegrass and 37 mg kg<sup>-1</sup> in five harvests of common bermudagrass.

The best balance between P inputs and crop removal was evident with a 'P-consistent' rate of 4.48 Mg ha<sup>-1</sup> litter, as M3-P decreased by about 62 mg kg<sup>-1</sup> in bermudagrass and 60 mg kg<sup>-1</sup> in ryegrass–bermudagrass during the study period. Additionally, M3-P was similar in the 0 and

4.48 Mg ha<sup>-1</sup> litter treatments at the end of study (Table 5). Based on conditions in the present study and providing P uptake and soil P are related linearly, the cessation of 4.48 Mg ha<sup>-1</sup> litter and harvesting hay for eight years would lower soil P to an acceptable agronomic level, i.e., slightly below 70 mg kg<sup>-1</sup>, above which the likelihood that environmental problems from high P soils may occur. With the adoption of a P-index for assessing risk of P loss to surface waters, the relationship between harvest removal and soil P levels provides much needed information on the safe and effective use of broiler litter as fertilizer. To the maximum extent possible, producers should apply litter at rates that balance P application and removal in the field, and export the harvested forage from the farm or feed it to animals that are not located on fields receiving manure. Because growers value litter as a N fertilizer source, they may be reluctant to reduce application rate or replace it with commercial fertilizer without adequate compensation (Chamblee and Todd 2002). The "Poultry Litter Clearinghouse" is a program managed by Mississippi Farm Bureau that serves as

**Table 5** Soil pH, total nitrogen (TN), water-extractable P (WEP), total P (TP) and selected Mehlich-3 extractable nutrient elements in April 1999 and in April 2004 at the end

Depth and litter rate	pH	TN (g kg <sup>-1</sup> )	WEP (mg kg <sup>-1</sup> )	TP (mg kg <sup>-1</sup> )	M3-P (mg kg <sup>-1</sup> )	M3-K (mg kg <sup>-1</sup> )	M3-Cu (mg kg <sup>-1</sup> )	M3-Zn (mg kg <sup>-1</sup> )
April 1999								
0–5 cm	5.8	2.34	ND	ND	232	230	13	16
5–15 cm	5.9	0.60	ND	ND	158	100	4	3
April 2004, 0–5 cm soil depth								
0	4.3 d <sup>a</sup>	1.58 d	13.6 d	782 c	198 d	118 c	18.2 d	17.4 c
4.48	4.4 cd	1.81 c	15.5 d	803 c	209 d	120 c	19.8 d	19.5 c
8.96	4.5 bc	1.98 bc	19.4 c	894 c	264 c	121 c	26.9 c	26.1 b
17.9	4.6 b	2.18 b	22.3 b	1,092 b	313 b	147 b	29.8 b	31.0 b
35.8	4.8 a	2.48 a	29.6 a	1,511 a	457 a	163 a	41.5 a	52.4 a
April 2004, 5–15 cm soil depth								
0	5.0	0.57	12.4 c	487 <sup>b</sup>	135 d	91	6.2 d	4.8 c
4.48	4.8	0.63	12.5 c	514	140 d	88	6.6 cd	5.2 bc
8.96	5.1	0.62	17.7 b	539	175 c	91	8.1 bc	6.2 ab
17.9	5.0	0.60	20.0 b	629	211 b	112	8.9 ab	6.9 a
35.8	5.0	0.63	24.6 a	760	246 a	98	10.4 a	7.6 a

ND no data

<sup>a</sup> Litter rate means ( $n = 8$ ) followed by a different letter are significantly different at the 5% level according to Fisher's protected least significant difference; otherwise, not significant

<sup>b</sup> Difference between litter rate means significant at 10% level of probability

an intermediary between broiler producers who wish to move litter off their farm and other growers, ideally outside the watershed, who wish to make use of its fertilizer value.

Analysis of M3-P within each sampling date found no significant effect of forage system ( $P > 0.10$ ) or its interaction with litter rate ( $P > 0.20$ ). While this result indicates that increased P uptake by ryegrass–bermudagrass did not translate to significant reductions in M3-P, this lack of integration has been encountered in other phyto-remediation studies (Novak and Chan 2002; Read et al. 2007). But we also observed that M3-P levels were elevated by about  $23 \text{ mg kg}^{-1}$  in ryegrass–bermudagrass when 0 or  $8.96 \text{ Mg ha}^{-1}$  litter was used (Table 4). These results should be viewed in the context of a large pool of P in this manure-impacted soil relative to the amount removed in the forage. Additionally, annual ryegrass is reported to have high root:shoot P concentration ratio, particularly at high external P supply (Pederson et al. 2002; Sharma and Sahi 2005). In greenhouse studies, Sharma and Sahi (2005) reported a 30% increase in phytase activity in roots of Marshall annual ryegrass when soil was amended with inorganic P ( $2.5 \text{ g kg}^{-1}$  soil). Studies with various cropped soils have shown 5–70% of dissolved organic P was hydrolyzed when incubated with phytase, or acid or alkaline phosphatase (Gburek et al. 2005). Plants having a high phytase activity in their roots can hydrolyze phytates, which account for a large proportion of unavailable soil P pool, and can thus deplete the excess P source more efficiently. While enhanced P uptake cannot be directly correlated with phytase activity in roots, the increased P removal by ryegrass–bermudagrass would suggest increased availability of orthophosphates in soil solution for plant uptake. The presence of organic P forms in soil and their conversion to inorganic form by phytase for bioavailability and plant uptake would explain the sometimes elevated M3-P levels in ryegrass–bermudagrass. McLaughlin et al. (2005) similarly reported double-cropping enhanced P removal by 22% in a swine-effluent spray field, but M3-P at 5–10 cm soil depth was greater than in a bermudagrass winter fallow system at the end of the study (25 vs.  $10 \text{ mg kg}^{-1}$ ). They attributed this difference to the release of ryegrass P following annual death and decay of the fibrous root system and to

movement of P into this layer by water percolating through channels left by roots.

In order to compare the effectiveness of both forage systems to reduce high soil P in a manure-impacted soil, a simple calculation (or normalization) was used to “credit” the ryegrass–bermudagrass system with the additional M3-P observed when  $0 \text{ Mg ha}^{-1}$  was previously applied (Table 4). This involved averaging M3-P across sampling dates and subtracting values for the control from the different litter treatments. Results showed M3-P in ryegrass–bermudagrass averaged  $3.5 \text{ mg kg}^{-1}$  at  $4.48 \text{ Mg ha}^{-1}$  litter and  $51.5 \text{ mg kg}^{-1}$  at  $8.86 \text{ Mg ha}^{-1}$  litter, and were less than the corresponding values for bermudagrass winter fallow (27.4 and  $55.2 \text{ mg kg}^{-1}$ ). Normalization of water-extractable P (WEP) similarly indicated larger reductions by the double-cropping system of about 2.0 and  $0.2 \text{ mg kg}^{-1}$  at litter rates of 4.48 and  $8.96 \text{ Mg ha}^{-1}$ , respectively, as compared to bermudagrass winter fallow (data not shown). A greater decline in M3-P and WEP relative to control has important water quality implications, because the soil surface is the most active zone of P detachment or P solubilization to cause increased runoff P losses to the environment for the long term (McDowell et al. 2001; Pierson et al. 2001).

#### Final soil chemical properties

Soil chemistry is the ultimate test of phytoremediation sustainability, as well as the safe and effective management of broiler litter. Soil pH decreased from 5.8 in 1999 to 4.8 in 2004 in all the litter treatments (Table 5), probably due to the acidifying effects of  $\text{NH}_4\text{NO}_3$  in surface soil, as litter N was replaced with commercial N. Sharma and Sahi (2005) reported decreased phytase activity and P uptake in annual ryegrass as pH decreased from 7.8 to 5.6 in an amended soil. As expected, the use of  $35.8 \text{ Mg ha}^{-1}$  litter significantly increased total N, WEP, total P, and Mehlich-3 extractable P, K, Cu and Zn in the 0–5 cm soil depth. Brink et al. (2008) reported apparent P recovery by bermudagrass of 4% when  $35.8 \text{ Mg ha}^{-1}$  litter was applied in spring of the current season’s growth. Clearly, the highest litter rate represents an increased risk of P loss to the environment due to poor use by plants on soil already very high in extractable P (McDowell et al. 2001; Gburek et al. 2005). The observed trends in soil P

following the cessation of litter application and results of Brink et al. (2008), Read et al. (2006) and Chamblee and Todd (2002) indicate the fertilizer value of litter nutrients could be more effectively captured by using the litter on fields with STP below the optimum range and selling any excess for use elsewhere.

When a P-consistent rate of  $4.48 \text{ Mg ha}^{-1}$  litter was used, M3-P in April 2004 (0–15 cm depth) was similar to  $0 \text{ Mg ha}^{-1}$  litter and somewhat lower than the initial value (163 vs.  $182 \text{ mg P kg}^{-1}$ ) (Table 5). This agrees with other studies (Evers 2002; Read et al. 2006; Brink et al. 2008) that  $4.48 \text{ Mg ha}^{-1}$  litter may be appropriate both agronomically and environmentally, because most of added P (Table 3;  $76 \text{ kg ha}^{-1} \text{ P}$ , equivalent to  $174 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ ) would be utilized by the forage crops; about 72% of the applied P was recovered by ryegrass–bermudagrass in the present study. Applying more than  $4.48 \text{ Mg ha}^{-1}$  litter significantly elevated WEP and Mehlich-3 extractable P, K, Cu and Zn in the 0–5 and 5–15 cm soil depths. Applying more than  $8.96 \text{ Mg ha}^{-1}$  litter elevated total P in the 0–5 cm soil depth ( $P < 0.05$ ), and a similar trend was evident in the 5–15 cm depth. Elevated Cu and Zn is consistent with the practice of adding micronutrients to poultry rations, their subsequent excretion in litter (Sistani et al. 2003), and low rate of removal in the harvested forage (Pederson et al. 2002; Read et al. 2007). Brink et al. (2001, 2004) reported hybrid bermudagrass removed only 1.0–1.5% of Cu and 7–13% of Zn, as compared to 15–27% of P that was applied in  $11.8 \text{ Mg ha}^{-1}$  litter to Savannah soil with long history of litter. In the present study and similar to M3-P, soil Cu and Zn were elevated at litter rates greater than  $4.48 \text{ Mg ha}^{-1}$  and micronutrient levels were consistently greater in 0–5 than the 5–15 cm soil depth (Table 5). Because Cu and Zn are associated closely with soil organic matter, they are generally retained in surface soil (Novak et al. 2004).

## Conclusions

Soils used for the application of poultry litter often have a high residual fertility, particularly in the case of P, which is relatively insoluble and has a long residence time in the soil. Water quality problems can occur if P enters the surface water in runoff. This

study compared the potential of two forage systems commonly grown in the southeastern USA, ryegrass–bermudagrass and bermudagrass winter fallow, to reduce the level of P in a manure-impacted soil previously amended with five rates of broiler litter. Forage biomass increased significantly as antecedent litter rate increased and annual P removal rates in the two systems ranged from 46 to  $74 \text{ kg ha}^{-1}$ , depending on litter rate and study year. Despite greater biomass yield and P uptake by ryegrass–bermudagrass, the level of M3-P in surface soil (0–15 cm) did not differ significantly between the tested systems, and may be related to the large mass of P in soil compared with P removal. Additionally, M3-P in soil not previously amended with litter was slightly elevated in the double-cropping system, possibly due to hydrolysis of organic P through enhanced phytase activity in ryegrass roots (Sharma and Sahi 2005). When a P-consistent rate of  $4.48 \text{ Mg ha}^{-1}$  litter was used, the combined harvests of ryegrass and bermudagrass removed  $55 \text{ kg ha}^{-1} \text{ P}$  and reduced M3-P levels by 13% annually (or about  $30 \text{ mg kg}^{-1}$  soil). The performance of bermudagrass was important in P removal from a manure-impacted soil and a double crop of ryegrass–bermudagrass removed the most P when rainfall distribution during summer was less than adequate for optimum bermudagrass yield.

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